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# Toxicity Studies of Poly Herbal Siddha Formulation Narasinga Rasayanam

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## Abstract

*Narasinga Rasayanam(NR)* is a poly herbal traditional *Siddha* preparation. The name *Narasingam* denotes *Serankottai* (Semecarpus anacardium) based formulation which is widely used in South India as traditional Siddha medicine for the treatment of peptic ulcer (*Gunmam*) and for regenerative conditions. It is more popular medicine and also in use for very long period but there is no documented data on its safety. The present study is aimed to investigate the acute and sub acute toxicity profile of *Narasinga Rasayanam(NR)* in healthy Wistar rats by OECD guidelines. In acute toxicity study, NR (2000mg /kg) was administered to the wistar albino rats and observed for 14 days. For Sub acute toxicity study the drug NR was administered with the dose of 144 mgm, 720 mgm, 1440 mgm for 28 days. The results of this study showed that the drug NR neither produced significant changes in the consumption of food and intake of water, nor affected biochemical parameters, haematological parameters and there was no change in histopathology studies. Finally this study results concluded that, the drug NR at a dose of 2 g/kg was found to be safe as a potent Anti-Ulcer agent in Wistar rats.

**Keywords:** Toxicity study; Siddha Medicine; *Narasinga Rasayanam.*

## Introduction

*Siddha* medicine, one of the oldest traditional medical system of India, uses metals and minerals as drugs extensively in addition to plants and animal products to treat several diseases.(1)

Herbs are playing major role in *Siddha* and other AYUSH system of medicine. The use of herbal medicines particularly in *Siddha*, *Ayurvedha* and *Unani* medicines for healing purpose has been increasing in which plants are mostly used for medicine preparation. Medicinal plants have long been considered as valuable sources of medicine for treating variety of diseases and ailments. The increase in the indiscriminate use of plant extracts is further aggravated by the belief that plants are safe simply because they are natural in origin (2). The use of medicinal plants for healing purposes has been increasingly popular as they are believed as beneficial and free of side effects(3).

Medicinal plants are often used without satisfactory demonstration of their pharmacological activities. During the past few years it is observed that, the adverse effects of phytomedicines, as well as its adulteration, toxicity, and drug interaction are common problems related to public health[4]. The reorganization and acceptability of herbal medicines has been limited due to lack of defined chemical characterization, dose regimen and adequate toxicity data to determine their safety [5]. The consumption of medicinal plants as conventional medications or/and as curatives may cause adverse toxicological effects to human health.

However, the rationale for the utilization of medicinal plants has rested largely on long term clinical experience with little or no scientific data on safety. With the upsurge in the use of herbal medicines for various diseases, toxicological investigation of medicinal plants is imperative based on the need to validate their folkloric usage [6].

*Narasinga Rasayanam*[7] is one of the poly herbal Siddha preparation which is being used for *Gunmam* (Peptic ulcer), *Paandu* (Anaemia), 18 types of *Kuttam* (Skin diseases), *Magotharam* (Ascites) and *VaiyitruKatti* (Abdominal tumour) but there is no scientific safety data on it, at the same time there is sufficient safety data are available for the ingredients of *Narasinga Rasayanam.* The ingredients of this Siddha formulation are, Kodiveli (Root of *Plumbago zeylanica*),Serankottai (*Semecarpus anacardium*), Thannervittan Kizhangu (*Asparagus racemosus*), Nerunjil (*Tribulus terrestris*) and Nilapanai Kizhangu (*Curculigo orchioides*).

The traditional healers and Siddha physicians are using this drug, clinically its working well in the above said conditions. For justifying the safety of this drug, there is no evidence of toxicological data. So the present study is aimed to evaluate the toxicological effect of *Narasinga Rasayanam* through acute and sub acute toxicity studies.

## Materials and Methods

### *Narasinga Rasayanam* Preparation:

### Ingredients:

Kodiveli Ver (*Plumbago zeylanica*) - 560g
Serankottai (*Semecarpus anacardium*) -560g
Thannervittan Kizhangu (*Asparagus racemosus*) -2240g
Nerunjil (*Tribulus terrestris*) - 280g
Nilapanai Kizhangu (*Curculigo orchioides*) - 700g
Palm jiggery - 875g
Honey - 560g
Cow's Ghee - 280g

### Procurement of Raw Drugs:

The raw drugs were procured from a well reputed country shop in Parrys corner, Chennai and authenticated by Botanist, National Institute of Siddha. All the herbal ingredients were purified (detoxification) by the suitable method specified in the Siddha literature.

### Method of Preparation:

*Kodiveli, Serankottai, Thanneervittan, Nerunjil, Nilappanai Kizhangu* were purified and dried in sun shade, then it was made into fine powder separately and finally mixed together. After that in the mixture of all herbal powder, the ghee, honey and palm jiggery were added little by little and ground in a *Kalvam* (stone mortar) until it attained waxy consistency. Then it was stored in an air tight container.

### Experimental Animals:

Healthy out bred Wistar Albino Rats of either sex weighing about 120 – 160 g were selected. The female rats obtained were nulliparous and non pregnant. All the animals were properly maintained and strictly following the "Guidelines on care and maintenance of laboratory animals" that have been framed by the Committee for the

Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Forests and Climate Changes, Government of India. The animals received RO water ad libitum and fed with Rodent pellet diet. Before the induction of toxicity study, all the animals were acclimatized for seven days. The study protocol has got approval from Institutional Animal Ethical Committee approved Number: IAEC/XLIX/14/CLBMCP/2016.

## Acute toxicity Study: [8]

This study was carried out by following the procedure with the starting dose of 2000 mg/kg body weight of test drug mentioned in OECD 423 guideline; six female rats were randomly selected and acclimatized prior to the study. Each selected animal was kept in separate poly propylene cage and marked with picric acid on the fur for identification. The rats were fasted overnight before administering the test drug. After the administration of test drug, the rats were deprived of feed for 16 h and water was not allowed for initial 3 h. The study was conducted initially with the starting dose of 2000 mg/kg administering in three rats and observed for mortality. As there was no mortality, three more rats were subjected to the study with the same dosage of test drug. The test drug *Narasinga Rasayanam* was administered through oral gavage suspended in the lukewarm water as single dose. The rats were observed for mortality, behavioural changes and clinical signs of toxicity for half an hour once in first four hours after dosing and thereafter periodically up to 14 days on same time of each day.

## Sub-acute toxicity study: [9]

This study was carried out by following OECD guidelines adopted for the testing of chemicals – 407. In the literature *Sarabenthira Vaithiya Muraigal -Gunma Roga Sigitchai*, the human intended dosage for NR was recommended as 4g twice a day (8000 mg/day). On the basis of body surface area conversion against human dose, 8000mg/kg/day dosage of NR was calculated for rat (Paget and Barnes, 1964). In the present study, three doses of NR of 144 mg/kg/day (Low dose), 720 mg/kg/day (Mid dose) and 1440 mg/kg/day (High dose) were selected for administration. Wistar Albino rats of both sex were randomized into four groups of ten animals each (5 males, 5 females). Group I received a vehicle (distilled water) and served as control group. Group II, III and IV served as low, mid and high doses of NR respectively. All the test substances were administered once daily via oral route through gastric gavage for 28 days. All the rats were observed daily for mortality, morbidity and abnormal clinical signs on each day for the same time. The body weight change, water and food consumption of each rat was monitored once a week. At the end of 28 days treatment, live rats were fasted over night and on the 29 $^{th}$ day under light chloroform anaesthesia, blood were drawn using capillary tube from the retro orbital sinus and added into a tube with potassium EDTA and a tube without anticoagulant. The haematological parameter tests such as Haemoglobin (Hb), Red Blood Cell count (RBC), White Blood Cell count (WBC), Differential count - Lymphocyte, Monocyte and Granulocyte, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Platelet (Plt) were done in the EDTA mixed blood samples using Erba Mannhein® haematology analyser. The blood samples without anticoagulant were used for estimating biochemical parameters such as Glucose, Cholesterol, Triglyceride (TG), Protein, Urea, Creatinine, Bilirubin, Serum glutamic-oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT) and Alkaline Phosphatase (ALP) using Erba system Pack kits in Fully Automated Biochemistry analyzer (Transasia EM 360). After withdrawal of blood, all the rats were sacrificed for gross necropsy and histopathological study. Organs including brain, lungs, heart, liver, kidney and spleen were studied for gross necropsy and weighed for calculating relative organ weight. Histopathological studies on brain, liver, kidney, lungs, heart and spleen were carried out for control and high dose group. The tissues of collected organs were fixed in 10% Neutral buffered formalin for 24 h. The tissues were trimmed, embedded in molten paraffin wax and sectioned (4-5 microns thickness) using rotary

microtome. The sections were floated in hot water and placed in the glass slide. The slides were stained with Haematoxylin and Eosin (H&E), mounted in DPX and examined under light microscope (Singh and Sulochana, 1997).

### Statistical analysis:

All data were expressed as mean ± standard deviation (SD). The test groups were compared with control group for testing significance and done by One-way Analysis of Variance (ANOVA) followed by Dunnett Multiple Comparisons Test using GRAPH PAD INSTAT version 3 software programs. Values of $p<0.05$ were considered significant.

## Results

### Acute Toxicity study:

The safety profile of this preparation *Narasinga Rasayanam* in acute and sub acute tests are not determined yet, this research showed the safety of this Siddha medicine *Narasinga Rasayanam* in two models of toxicity assessment. It is deemed important to evaluate the toxicity effect of herbal preparation in order to increase the confidence in their safety to humans, particularly for use in the development of nutraceuticals and pharmaceuticals. To our best knowledge, this is the first study reported the toxicity effects of *Narasinga Rasayanam* in rats. The acute toxicity study does not show any toxic symptoms, changes in behaviour or mortality at 2000 mg/kg doses. Through-out the 14 day periods all animals were found to be healthy with no changes in their skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system and as well as somatomotor activity and behavioural patterns. No gross pathological abnormality in the organs was found even at this high dose. On the basis of above observations, this preparation has anticipated having an LD50 higher than 2000 mg/kg bodyweight which is not hazardous in acute doses. [10] The results of observation in acute toxicity study have been tabulated. (Table.No-1).

**Table: 1: Effect of *Narasinga Rasayanam* in Acute Toxicity study**

| No | Dose mg/kg | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Control | + | - | - | + | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2. | 2000mg | + | - | - | + | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

1. Alertness
2. Aggressiveness
3. Pile erection
4. Grooming
5. Gripping
6. Touch Response
7. Decreased Motor activity
8. Tremors
9. Convulsions
10. Muscle Spasm
11. Catatonia
12. Muscle relaxant
13. Hypnosis
14. Analgesia
15. Lacrimation
16. Exophthalmos
17. Diarrhoea
18. Writhing
19. Respiration
20. Mortality

**[(+) indicates the presence (-) indicates the absence].**

**Sub-acute Toxicity Study:**

In sub acute toxicity study, it appeared that the *Narasinga Rasayanam* at the doses (144mg/kg, 720 mg/kg, & 1440 mg/kg) did not produce any marked changes in both male and female rats, as evidenced by the absence of toxic symptoms, no changes in water/food ingestion, or weight gain. Normal organ weight revealed that the study drug did not produce organ swelling, atrophy or hypertrophy. Feed and water consumption of treated groups were found not to be significantly affected or changed in both sexes compared to the lukewarm water treated rats. Consumption of toxic substances effects at least a minimal reduction in body weight gain and internal organs weight [(11)]. During the study, the treated test groups gained weight gradually in respective to the feed intake similar to the control group. The animal's body weight was also not altered by *Narasinga Rasayanam* treatments (Table- 2).

**Table: 2: Effect of *Narasinga Rasayanam* on Body weight (g) - 28 days Repeated dose oral toxicity study.**

| Group | 1st Day | 7th Day | 14th Day | 21st Day | 28th Day |
|---|---|---|---|---|---|
| **Control** | 140.86±1.71 | 146.72±1.3 | 151.88±1.9 | 156.92±1.9 | 162.94±2.11 |
| **Low Dose** | 141.7±2.56 | 147.34±1.8 | 153.1±1.78 | 158.24±2.9 | 163.22±2.68 |
| **Mid Dose** | 142.22±1.66 | 148.5±2.06 | 153.9±2.12 | 161.04±2.0 | 161.04±2.07 |
| **High Dose** | 142.42±1.78 | 150.14±1.5 | 156.22±2.1 | 162.4±2.25 | 170.2 ±2.06 |
| **P value (p)*** | NS | NS | NS | NS | NS |

NS- Not Significant, ** (p > 0.01),*(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett‟s test)

The changes observed in blood parameters analysed in laboratory animals provide the evidence of risk of toxic effects on haematological system (Olsan et al., 2000). The haematological profile of the treated and control groups were presented in the (Table-3). White blood cell parameters tested such as Lymphocytes, Monocytes and Granulocytes in both male and female rats showed no significant differences in relation to the control group. However, the significant differences noted in the parameters were lies within normal physiological limits indicated that *Narasinga Rasayanam* did not affect haematopoiesis or leukopoiesis in rats and that suggested *Narasinga Rasayanam* did not produce any toxicity in the blood forming organs affecting the haematopoietic indices.

**Table: 3: Effect of *Narasinga Rasayanam* on haematological parameters - 28 days Repeated dose oral toxicity study.**

| Parameter | Control | Low dose | Mid dose | High dose | P value |
|---|---|---|---|---|---|
| **Haemoglobin (g/dl)** | 16.7±0.71 | 16.60±0.24 | 16.5±0.23 | 16.82±0.16 | **NS** |
| **Total WBC (109/L)** | 10.81±0.32 | 10.64±0.21 | 10.54±0.42 | 9.60±1.12 | N.S |
| **Neutrophils (%)** | 31.12±0.01 | 31.02±0.12 | 32.11±1.22 | 33.02±6.21 | N.S |
| **Lymphocyte (%)** | 72.12±1.24 | 72.12±1.32 | 73.10±2.34 | 73.20±2.44 | N.S |
| **Monocyte (%)** | 0.9±0.02 | 0.9±0.01 | 0.9±0.04 | 0.9±0.03 | N.S |
| **Eosinophil (%)** | 0.5±0.03 | 0.5±0.04 | 0.5±0.05 | 0.5±0.08 | N.S |
| **Platelet (109/L)** | 680.17±3.13 | 682.41±4.12 | 682.13±2.02 | 684.10±2.34 | N.S |
| **Total RBC (1012/L)** | 8.42±0.12 | 8.46±0.53 | 8.49±0.44 | 8.74±0.46 | N.S |
| **PCV%** | 42.12±0.2 | 42.62±1.02 | 43±1.20 | 44.40±2.10 | N.S |
| **MCHC g/dL** | 34.5±1.20 | 34.2±1.10 | 34.8±1.70 | 34.33±1.30 | N.S |
| **MCV fL(μm$^3$)** | 58.2±4.02 | 59.2±1.10 | 58.9±1.40 | 58.8±1.20 | N.S |

N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett‟s test)

The biochemical profiles of the treated group were presented in the (Table- 4). The biochemical parameters level indicates physiological condition. The increase and decrease of biochemical parameters can convey indications regarding toxicity of specific organs. In the present study, biochemical parameters were estimated particularly SGOT, SGPT, ALP, creatinine, total cholesterol and total protein were tested and the analysis showed that there was no significant differences in parameters level of the rats treated with *Narasinga Rasayanam* compared to the control. Although there is a slight decrease or increase in the level of biochemical parameters in treated rats compared to the control, these values were still within the normal range. The biochemical findings suggested that the administration of *Narasinga Rasayanam* did not cause any toxicological effect. Morphological examination on the vital organs, liver, kidney and heart as well revealed no treatment-related changes due to the administration of *Narasinga Rasayanam* in the animals. The liver and kidney were studied extensively for histopathology observation because of their primary function to expel toxins that results from body's metabolism of food, drug or any other substances that was being consumed. In sub-acute toxicity studies, the rats treated with the *Narasinga Rasayanam* showed normal architecture of the liver and kidney. There is no evidence of lesion due to toxic effect of *Narasinga Rasayanam* in the liver and kidney. (Figure no. 1 - 4).

**Table: 4: Effect of *Narasinga Rasayanam* on Biochemical parameters - 28 days Repeated dose oral toxicity study.**

| Parameter | Control | Low Dose | Mid Dose | High Dose | P value |
|---|---|---|---|---|---|
| **Glucose (mg/dl)** | 125.11±3.2 | 125.12±2.10 | 126.10±13.08 | 128.12±4.2 | N.S |
| **Cholesterol (mg/dl)** | 120.16±1.20 | 120.25±1.30 | 122.60±1.18 | 123.24±1.30 | N.S |
| **Triglyceride (U/L)** | 54.16±1.52 | 54.12±1.42 | 56.15±1.23 | 56.16±1.23 | N.S |
| **Protein (g/dL)** | 6.2±0.04 | 6.2±0.11 | 6.2±0.10 | 6.4±0.46 | N.S |
| **Urea (mg/dl)** | 26.70±0.19 | 26.50±0.26 | 27.16±1.28 | 27.68±1.24 | N.S |
| **Creatinine (mg/dl)** | 0.22±0.02 | 0.21±0.04 | 0.22±0.05 | 0.24±0.07 | N.S |
| **Bilirubin (mg/dl)** | 0.07±0.01 | 0.07±0.02 | 0.07±0.01 | 0.07±0.03 | N.S |
| **SGOT (U/L)** | 81.14±1.63 | 81.31±0.02 | 82.01±1.24 | 82.64±1.63 | N.S |
| **SGPT (U/L)** | 78.12±1.08 | 78.21±1.24 | 78.14±1.26 | 77.68±0.01 | N.S |
| **ALP(U/L)** | 119.21±3.16 | 119±32.10 | 119±12.14 | 120.03±8.32 | N.S |

NS- Not Significant, **(p > 0.01), * (p >0.05) , n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett‟s test)

**Control - Liver -NAD**

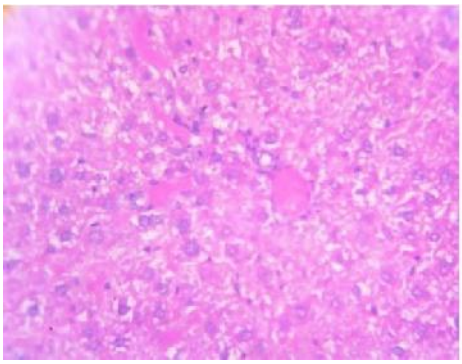

**Figure: 1**

**High Dose - Liver- NAD**

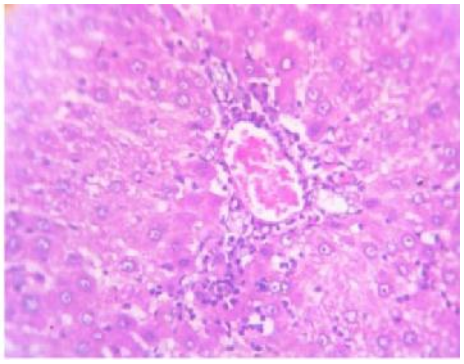

**Figure: 2**

**Control - Kidney - NAD**

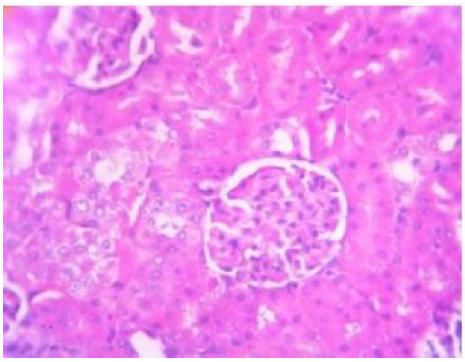

**Figure: 3**

**High Dose - Kidney- NAD**

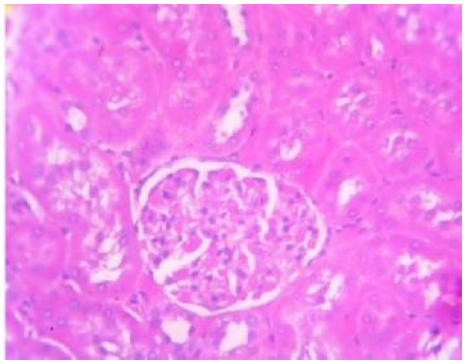

**Figure: 4**

## Conclusion

The study drug *Narasinga Rasayanam* was found to be nontoxic when oral acute and sub acute (28 -days repeated dose) toxicity studies in rats were performed. No signs of toxicity were observed in the histopathological studies. This study data suggests that the oral administration of *Narasinga Rasayanam* did not induce any toxic effect at 1440 mg/kg/day dose in rats, and this stands as an assurance of safe usage at its desirable human intended therapeutic dosage of 4 gm two times a day in the practice of Siddha medicine.

## References


1. Ilango . B et.al. Histopathological Studies of the effect of Naga Parpam, A Zinc based drug of Siddha Medicine, In Rats; Journal of Cell and Tissue Research Vol. 9(2) 1869-1873 (2009.
2. Anoja Priyadarshani Attanayake et al. Efficacy and toxicological evaluation of *Coccinia grandis* (Cucurbitaceae) extract in male Wistar rats; Asian Pacific Journal of Tropical Disease; 2013; 3(6): 460-466.
3. da Costa Lopes L, Albano F, Augusto Travassos Laranja G,Marques Alves L, Fernando Martins e Silva L, Poubel de Souza G,et al. Toxicological evaluation by in vitro and in vivo assays of an aqueous extract prepared from *Echinodorus macrophyllus* leaves. Toxicol Lett 2000;116:189-98.
4. Saude-Guimaraes DA, Fernanda CF, Andrea GG, Claudia MC, Maira RD, Tania T et al. Toxicological evaluation of ethanolic extract of Lychnophora trichocarpha, Brazilian arnica, Rev. Bras. Farmacogn. Braz. J. Pharmacogn. 2012; 22(5): 1104-10.
5. Gautam MK, Goel RK. Toxicological Study of Ocimum sanctum Linn Leaves: Hematological, Biochemical, and Histopathological Studies. Journal of Toxicology, 2014; 1-10.
6. Anoja P. Attanayake et al., Toxicological investigation of *Spondias pinnata* (Linn. F.) Kurz. (Family: Anacardiaceae) bark extract in Wistar rats; International Journal of Green Pharmacy | January-March 2015.
7. Vasudeva Sasthiri.K, Dr.Venkada Rajan.S. Sarabenthira Vaithiya Muraigal-Gunma Roga Sigithchai, Third edition, Saraswathi Mahal, Pg.64, 65.
8. OECD. Guideline Number 423 for the Testing of Chemicals: Revised Draft Guideline 423 (Acute Oral Toxicity). Paris, France: OECD; 2000.
9. OECD guideline number 407 for the testing of chemicals , Paris, France

10. Sreemoy Kanti Das.et.al. Toxicological Investigation of Ethanolic Extract of *Epipremnum aureum* in Rodents; Journal of Applied Pharmaceutical Science; Vol. 5 (Suppl 2), pp. 057-061, 2015.
11. Teo S et.al. 90 days oral gavage toxicity study of d-methylphenidate and d,l methylphenidate in Sprague Dawley rats. Toxicology, 2002; 79:183–196 .

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